BACTERIAL CONTAMINATION OF SURFACES AND EQUIPMENT AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF POTENTIALLY PATHOGENIC BACTERIA IN NEWBORN UNIT AT KENYATTA NATIONAL HOSPITAL

BY

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DECLARATION

This dissertation has not been submitted for any degree in other universities.

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DEDICATION

This study is dedicated to my daughters Adol and Abuk for understanding my unavailability for them sometimes, to my amazing and wonderful parents Mary Mohamed Mingi and Chan Malual Chan for their prayers, words of support and encouragement, and to the neonates who were admitted to the newborn unit of Kenyatta National Hospital during sample collection.

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AST	Antimicrobial susceptibility testing
CLSI	Clinical and Laboratory Standards Institute
ESBL	Extended-spectrum beta-lactamase
HAI	Healthcare-associated infection
IPC	Infection prevention control
KNH	Kenyatta National Hospital
LBW	Low birth weight
MRSA	Methicillin-resistant *Staphylococcus Aureus*
NBU	Newborn unit
NICU	Neonatal intensive care unit
UNITID	University of Nairobi Institute of Tropical and Infectious Diseases
UON	University of Nairobi

DEFINITIONS

Neonate: Infant aged 0-28 days.

Healthcare-associated infections in newborns: Infections acquired by the newborn after birth in a health care facility between 72 hours and 7 days of admission.

Preterm birth: Baby born alive before 37 weeks of gestation.

ABSTRACT

BACKGROUND

Nosocomial infections in newborn units pose a great challenge to health care systems. They have been linked to contaminated surfaces and equipment in neonatal wards. The sources and spread of the infections are mainly attributed to contamination of fomites within the hospital environment.

OBJECTIVE

To investigate the bacterial contamination profile of surfaces and equipment in the newborn unit (NBU) of Kenyatta National Hospital (KNH) and determine the antimicrobial susceptibility pattern of selected potentially pathogenic bacteria, which include *E. coli, S. aureu*s, Coagulase-negative staphylococci (CoNS), *K. pneumoniae, and P. aeruginosa.*

METHODS

A cross-sectional study was conducted in the NBU of KNH. Samples from surfaces and equipment were systematically collected from NBU until the required sample size was obtained. All the steps of sample collection and inoculation were carried out using aseptic techniques and transported to the Microbiology Laboratory of the University of Nairobi (UON) within 1- 2 hours of collection for analysis. Samples were cultured on selective and non-selective media. Phenotypic identification of the isolates was based on colonial morphology, gram staining, and biochemical tests. Antimicrobial susceptibility testing for selected bacteria was determined using the Kirby-Bauer disk method. Univariate and bivariate analyses were done using IBM® SPSS® software version 21.0 and data were presented in tables and graphs.

RESULTS

A total of 580 swabs were collected from surfaces and equipment in six different NBU locations/rooms. Following inoculation on the culture plates, 273 (54%) swabs showed growth. The majority of the positive bacterial cultures, 137/273 (50.2%), were coagulase-negative staphylococcus (CoNS). Others were: *Klebsiella pneumoniae,* 119/273 (43.6%); *Escherichia coli* 16/273 (5.9%), and *Pseudomonas aeruginosa* 1/273 (0.4%). Equipment and

surfaces with abundant growth included cots 55/273 (20%), radiant warmers 51/273 (19%), oxygen masks 46/273 (17%), incubators 16/273 (6%), desk surfaces 29/273 (11%), sinks 24/273 (9%), door handles 17/273 (6%) and taps 16/273 (6%). Most of the isolates were highly susceptible to meropenem, amikacin, and imipenem (70-100%) but resistant to penicillin, clindamycin, and vancomycin (45-100%).

CONCLUSION AND RECOMMENDATIONS

The study determined that newborn environmental surfaces and equipment at the Kenyatta National Hospital were contaminated with potentially pathogenic bacteria including CoN*S, K. pneumoniae,* and *E. coli.* The majority of the isolated bacteria were sensitive to meropenem, imipenem, and amikacin. All bacteria isolated had high resistance to penicillin, vancomycin, and clindamycin. The identified potentially pathogenic bacteria isolated from the NBU could be the source of infections to preterm and sick term neonate infants. Therefore, this study recommends improved compliance with infection control practices (hand hygiene, sterilization, and disinfection of patient-care items, devices, and environmental infection prevention and control) in the NBU at KNH.

Chapter 1

1.1 Background

Nosocomial infections play a significant role as the main cause of morbidity and mortality in hospitalized newborn infants (Kliegman *et al.,* 2016). Severe infections in neonates are among the main causes of the high mortality rates in children (Ateka, Songok, and Nyandiko, 2020). Most nosocomial infections occur in preterm or term infants who require intensive care. Bacteremia and neonatal sepsis are some of the diseases due to nosocomial infections in neonates, accounting for about 70% of hospital-acquired infections (Ateka, Songok, and Nyandiko, 2020; Kumar *et al.*, 2018). The prevalence of neonatal sepsis in the newborn unit (NBU) at Kenyatta National Hospital (KNH) in 2020 based on data collected from 196 neonates and their mothers admitted to the facility was estimated to be 28.6% (Okube and Komen, 2020). Other implicated diseases include meningitis and pneumonia (Levine, 2018), each is responsible for about 10% of nosocomial infections in neonates (Kumar *et al.*, 2018). Globally, more than a third of newborn deaths are due to severe infections, which are mostly hospital-acquired (Levine, 2018). The diseases contribute to the high neonatal mortality in Kenya since neonates with severe infections die at a disproportionately high rate.

The commonest risk factors for nosocomial infections in infants are prematurity, frequent use of antibiotics, low birth weight (Ramasethu *et al.,* 2017), parenteral nutrition (Kliegman *et al.,* 2016), endotracheal tubes, indwelling vascular catheters, alterations in the skin and/or mucous membrane barriers, prolonged hospital stay, and ventricular shunts. (Hewitt *et al.*, 2013; Lefrak *et al.,* 2016). A history of an invasive procedure is a significant risk factor for nosocomial bacteremia and sepsis (Okube and Komen, 2020). Peripheral vascular catheterization (PVC) and mechanical ventilation (MV) are some of the main interventions that may introduce nosocomial infections in neonates (Kumar *et al.*, 2018). PVC is associated with a disproportionately high risk of nosocomial infections compared to MV; it was 15.39 higher in the study by Kumar *et al.* (2018).

Evidence-based preventive strategies and interventions targeting the risk factors can substantially reduce nosocomial infections in neonates (Levine, 2018). Identifying fomites contaminated with causative bacteria can help in identifying the appropriate preventive interventions. For example, optimal aseptic techniques can be applied during invasive procedures to prevent bacteremia and sepsis (Okube and Komen, 2020).

Bacteria that cause nosocomial infections in neonates include *Escherichia coli, Pseudomonas aeruginosa,* coagulase-negative *staphylococci* (CoNS)*, Staphylococcus aureus,* and *Klebsiella pneumoniae* (Ramasethu *et al.,* 2017; Kliegman *et al.,* 2016). In a cross-sectional study done at Moi Teaching and Referral Hospital, the other national referral hospital in Kenya, *Klebsiella spp.* comprised 46.4% of the 151 bacteria isolates from 141 neonates with neonatal sepsis enrolled in the study. CoNS was the second commonest isolate at 27.8% (Ateka, Songok, and Nyandiko, 2020).

Some of the causative bacteria are becoming antibiotic-resistant. For example, a cohort study conducted among neonates admitted to a Kilifi Hospital in Kenya found that up to 55% (238/510) neonates acquired extended-spectrum β-lactamase–producing Enterobacterales (ESBL-E) carriage during hospitalization (Kagia *et al.*, 2019). In the study by Ateka, Songok, and Nyandiko (2020), the isolated *Klebsiella spp* were resistant to ceftriaxone, cefotaxime, and gentamicin. Methicillin-resistant *Staphylococcus aureus* (MRSA), which are commonly implicated in nosocomial infections were also detected in the samples. Therefore, the bacteria causing nosocomial infections are also spreading antimicrobial resistance.

According to a 2018 report by the World Health Organization, 2.5 million children died in the first twenty-eight days of life globally. The deaths among newborns contributed to the high under-five mortality rate; 47% of the deaths were among newborns. Sub-Saharan Africa had the highest neonatal mortality of 28 deaths per 1000 live births, which is very high compared to the global average of 17 deaths per 1000 live births (UNICEF, 2020). Regions like Europe and North America record only 3-4 deaths per 1000 lives. Even Nothern Africa has substantially lower neonatal mortality rates compared to Sub-saharan Africa, with 16 deaths per 1000 live births.

In lower and middle-income countries, preterm/ prematurity is the leading cause of neonatal deaths followed by birth asphyxia, and infections (Kliegman *et al.,* 2016; UNICEF, 2020). It is estimated that the neonatal mortality rate in low- and middle-income countries is 20 per 1000 live births, compared to 3 per 1000 live births in high-income countries. Infection contributes to 17% of deaths in sub-Saharan Africa as compared to 6% in developed countries. In Kenya infection is the third leading cause of neonatal death and the neonatal mortality rate is 22 deaths per 1000 births (Okube and Komen, 2020).

Neonates acquire infections either from health care providers in hospitals, the mother or from inanimate sources such as contaminated equipment (Kliegman *et al.,* 2016). The rate of occurrence of hospital-acquired infections ranges from 6% to 50%. Developing countries such as Kenya report higher rates. Nosocomial infections are common in the neonatal intensive care unit (NICU) environment because there are multiple fomites where bacteria can attach to become sources of infections (Kumar *et al.*, 2018).

Studies have reported that contaminated medical equipment and inanimate surfaces are associated with neonatal intensive care unit acquired infections (Cason *et al.*, 2021; Bhatta *et al.*, 2021; Baek *et al.*, 2020b). The multiple invasive diagnostic and therapeutic procedures in the neonatal intensive care unit (NICU) such as PVC and MV present risks for nosocomial infections among the neonates (Kumar *et al.*, 2018). Surfaces in the NBU that healthcare professionals get into contact with when administering the procedures can be contaminated with causative agents of nosocomial infections, thus introducing them into the neonate's body.

In Kenya, a study done at Mbagathi Hospital to characterize bacterial contaminants in surgical and newborn unit environments concluded that the rate of bacterial contamination was 18%. CoNS emerged as the predominant contaminants at 13% (Kamwati *et al.*, 2021). A study conducted at KNH's NBU only focused on neonatal sepsis (Beletew and Kassie, 2019). It did not investigate the causative agents of sepsis, which could be various bacteria, viruses, and fungi. They also did not establish whether environmental contaminants could be the source of infections leading to neonatal sepsis.

Despite the burden of nosocomial infection and its related morbidity and mortality, there is a scarcity of data on the hospital environment as a potential source of NBU contaminations. Besides, nosocomial infections are diverse and transient; KNH's NBU may be having a burden of nosocomial infections caused by a unique combination of pathogens that may have even changed over time. Therefore, it is important to investigate the current potential bacterial contaminants on various fomites in the NBU and determine their antimicrobial susceptibility profile to aid in the identification of appropriate interventions.

1.2 Literature review

1.2.1 Introduction

Nosocomial infection is not present at the time of admission; it is acquired while receiving medical care in a health facility (Salamati *et al.*, 2006). Neonates in the newborn unit /neonatal intensive care unit (NBU/NICU) are particularly susceptible to nosocomial infections because of the immaturity of their immune systems. The infected neonates are at a high risk of death. Some of the survivors of neonatal infections suffer neurocognitive disorders (Hewitt *et al.*, 2013). The common nosocomial infections in neonates admitted in NBU/NICU include conjunctivitis, meningitis, sepsis, bacteremia, pneumonia, and osteomyelitis (Ramasethu, 2017).

The source of infections in NBU/NICU is mainly through direct contact of the neonates with asymptomatic infected health care workers or parents (Kliegman *et al*.,2016; Kandwal *et al.*, 2019; Bitew, Gidebo, and Ali, 2021). Additionally, infections can be acquired indirectly via contaminated inanimate hospital objects including medical and non-medical equipment (Okolo *et al.*, 2016; Kliegman *et al.,* 2016; Kamwati *et al.*, 2021). According to Kweyu, Omwenga, and Maiyoh (2021), emergency scenarios such as the urgent need for resuscitation to address breathing challenges during uncommon complications of labor may lead to contamination of the equipment used. The urgency to save the neonate's life results in protocol deviations from strategies for aseptic procedures, hence introducing potentially-pathogenic bacteria and other microorganisms into the neonate's body and causing sepsis. The condition may start as pneumonia-like respiratory distress, then complicate into neonatal sepsis (Kweyu, Omwenga, and Maiyoh, 2021).

1.2.2 Fomites

Various inanimate objects have been described to play a role in nosocomial infections (Hewitt *et al.*, 2013; Russotto *et al.*, 2015). Devices in the intensive care units are commonly implicated as sources of nosocomial infections (Tauhid *et al.*, 2017). The equipment used in the alternative feeding of neonates who cannot breastfeed well may be contaminated and introduce infectious agents into the neonate (Kweyu, Omwenga, and Maiyoh, 2021). Other fomites that are probable causes of infection include an intravenous cannula, endotracheal tubes, suction catheter, and oxygen masks (Tauhid *et al.*, 2017).

When cultured, swabs of the probable fomites in the study by Tauhid *et al.* (2017) grew microorganisms whose identities and abundance were similar to the ones for microorganisms isolated from the culture of neonates' blood in the same NBU. The contaminated fomites become the sources of infectious agents that cause ventilator-associated pneumonia, central line-associated bloodstream infections, and catheter-associated urinary tract infections, among other infections (Tauhid *et al.*, 2017).

The hospital surface environment is a reservoir for nosocomial pathogens (Weber *et al.,* 2013). Pathogens in aerosols that are released when neonates, their mothers, or healthcare professionals sneeze or cough in the NBU settle on the surfaces, making them reservoirs of the microorganisms (Joshi and Kaur, 2019). The surface environment has been estimated to contribute up to 20% of nosocomial infections (Weber *et al.,* 2013).

Medical devices and healthcare professionals harbor the microorganisms that contribute to the other 80% of nosocomial infections (Joshi and Kaur, 2019; Weber *et al.,* 2013). Using contaminated medical devices to manage the neonates introduces pathogens to them. Microorganisms transfer from healthcare professionals to neonates through contaminated hands (Joshi and Kaur, 2019).

1.2.3 Epidemiology of nosocomial infections

The rate of nosocomial infections is high in African countries, it is estimated to be as high as 14.8% (Odoyo *et al.*, 2021). In the USA, the prevalence of hospital-acquired infections (HAIs) is about 3.2%. In the European Union, 6.5% is the prevalence of nosocomial infections (Sikora and Zahra, 2021). Given that surveillance systems for nosocomial infections are lacking especially in developing countries such as most African countries, the African countries' rates could be underestimated (Sikora and Zahra, 2021). Therefore, African countries are disproportionately affected by HAIs compared to North America and European countries.

In Kenya, nosocomial infections are estimated to affect about 10% of patients at any given time (Ministry of Health, 2021). About 10-25% of hospital admissions are attributed to hospital-acquired infections (Ministry of Health, 2021). Kenya introduced infection prevention and control guidelines for health care services in 2010 to address hospital-acquired infections but they remain a threat to patient safefy and quality patient outcomes.

The implementation and practice of the IPC guidelines and strategies in hospitals remain challenging because of limited resources (Ministry of Health, 2021). For example, there is poor adherence to hand hygiene due to a lack of consistent running water in some facilities. Hospital surfaces remain contaminated with pathogens even after cleaning and disinfection because most facilities conduct a visual inspection only instead of biomonitoring to evaluate the cleaning procedures (Kamwati *et al.*, 2021); Odoyo *et al.*, 2021)

The mortality rate among patients affected by nosocomial infections is about 10% (Haque *et al.*, 2020). The length of stay for patients who get nosocomial infections prolongs (Ministry of Health, 2021). The cost of care increases while health outcomes remain poor. Thus, HAIs cause a significant economic burden in society (Ministry of Health, 2021).

In Africa, studies have shown that healthcare providers have inadequate knowledge of infection surveillance and prevention. For instance, only 54-60% and 58% of healthcare providers in Ethiopia and Ghana respectively have adequate knowledge about infection prevention due to the unavailability of updated infection prevention guidelines and lack of adequate training on infection prevention (Assefa and Diress, 2020). According to the Ministry of Health (2021), Kenya needs to strengthen systems for sufficient surveillance of nosocomial infections.

1.2.4 Common bacterial causes of nosocomial infections in NBU

Gram-positive bacteria are commonly implicated in the causation of nosocomial infections. In the study by Kweyu, Omwenga, and Maiyoh (2021), the isolated bacteria were mainly gram-positive (35, 85.4%) while gram-negative bacteria were only 6 (14.6%). Gram-negative bacteria are also implicated as common causes of neonatal sepsis and other outcomes of nosocomial infections. Ghafoor *et al.* (2020) isolated more gram-negative bacteria (44 cultures, 57.9%) than gram-positive bacteria (32 cultures, 42.1%) from blood cultures obtained from 345 neonates. Tauhid *et al.* (2017) isolated 20 (86.9%) gram-negative cultures from the 23 positive blood cultures in their research.

1.2.4.1 Gram-negative bacteria

Klebsiella pneumoniae, one of the gram-negative bacteria, is an encapsulated bacterium commonly found inhabiting environment surfaces (soils and water surfaces). It has also been

isolated from medical devices. Naturally, *K. pneumoniae* colonizes human mucosal surfaces including the gastrointestinal tract and oropharynx (Paczosa and Mecsas, 2016). Neonatal intestines can therefore be a major reservoir of *K. pneumoniae* subsequently contaminating their surroundings. Further, *K. pneumoniae* can survive in the NICU environment for more than 2 years and can also re-emerge despite the implementation of infection control practices due to multidrug resistance. Healthcare workers, patients, and contaminated equipment have also been described as reservoirs of *K. pneumoniae. Klebsiella spp* is associated with systemic infections like neonatal septicemia, pneumonia, and meningitis especially in preterm neonates (Baek *et al.*, 2020). Ghafoor *et al.* (2020) identified *K. pneumoniae* as the second most common gram-negative cause of neonatal sepsis based on their blood culture results; nine (11.8%) of the positive blood cultures were *K. pneumoniae*. Tauhid *et al.* (2017) identified *K. pneumoniae* as the commonest pathogen in their samples since 8 (34.78%) of the 23 positive blood cultures had it.

Escherichia coli is a gram-negative bacterium that inhabits the lower gastrointestinal tract of humans and animals. *E. coli* has both commensal and pathogenic strains that cause a variety of human diseases including neonatal meningitis and sepsis (Lai *et al.*, 2021). It was the most abundant in the cultures isolated by Ghafoor *et al.* (2020), with 10 (13.1%) of the cultures showing it. *E. coli* was amongst the main bacteria isolated in the study by Kumar *et al.* (2018). Ateka, Songok, and Nyandiko (2020) also identified *E. coli* in 4 (2.6%) out of the 151 cultures. Tauhid *et al.* (2017) also detected *E. coli* in three (13.04%) of the 23 positive blood cultures.

Acinetobacter spp. are gram-negative coccobacilli that exist as free-living saprophytes in soil and water and some as commensals on the human throat, secretions, and skin. They were identified in eight (10.5%) of the positive cultures (Ghafoor *et al.*, 2020). It was the third commonest bacteria in the study by Tauhid *et al.* (2017) as it was present in 5 (21.73%) of the 23 positive blood cultures. *Acinetobacter baumanii* was the main gram-negative causative pathogen in the study by Kumar *et al.* (2018). Ten (6.6%) of the 151 cultures identified by Ateka, Songok, and Nyandiko (2020) were *Acinetobacter baumanii*, making it the third most prevalent bacteria isolated in the study.

Pseudomonas aeruginosa is mainly isolated from environments and has been shown to contaminate NICU environments, mainly from water sources within the hospital settings

(sinks, water taps, water baths) (Plecko *et al.*, 2017). Ghafoor *et al.* (2020) isolated *P. aeruginosa* from six blood cultures. Two (8.7%) of the 23 positive blood cultures in the study by Tauhid *et al.* (2017) had *P. aeruginosa*.

1.2.4.2 Gram-positive bacteria

Staphylococcus aureus is a gram-positive bacterium and a normal flora of human skin and mucus membrane. Neonates are first colonized by *S. aureus* during delivery, from either the healthcare workers or the inanimate objects in the nursery environment (Popoola and Milstone, 2014). *Staphylococcus aureus* is responsible for most infections seen in hospitalized preterm neonates due to contaminations from high-touch areas or inanimate objects (Romano-Bertrand *et al.*, 2014). Ghafoor *et al.* (2020) identified 28 *S. aureus* cultures from the 76 positive cultures, making it the commonest bacterial isolate in the study. *S. aureus*, more particularly MRSA, was the commonest isolate identified by Kumar *et al.* (2018). Ateka, Songok, and Nyandiko (2020) identified 7 (4.7%) *S. aureus* cultures from the 151 bacterial cultures grown. Only one (4.4%) of the positive blood cultures in the study by Tauhid *et al.* (2017) had *Staphylococcus spp*.

Coagulase-negative s*taphylococci* comprise the predominant microbiota on the skin and mucus membrane of humans; they are commonly implicated in nosocomial infections. Most CoN*S* can form biofilms that significantly contribute to their pathogenicity. Coagulase-negative *Staphylococci* have been recovered from blood cultures of sick and preterm neonates who have a central venous catheter (Becker, Heilmann, and Peters, 2014; Michels *et al.*, 2021). Ateka, Songok, and Nyandiko (2020) detected 42 (27.8%) CoNS bacterial isolates from the 151 bacterial cultures that grew.

1.2.5 Bacteria as surface contaminants

Previous studies focusing on environmental surface contamination isolated *Klebsiella* spp., *Staphylococcus* spp., *Escherichia* spp., and CoN*S* from health care facilities (M. Okolo *et al.*, 2016; Bhatta *et al.*, 2018). The distribution of bacteria varies in medical and non-medical equipment as determined in cultures of swabs of the medical equipment including radiant warmers, continuous positive airway pressure machines, oxygen masks, and incubators, and thermometers.

In several studies, equipment that come into direct contact with infant skin or mucus membranes were mostly colonized with *Enterobacteriaceae* and *Staphylococcus* spp. (Rastogi *et al.*, 2012; Artelt *et al.*, 2018; Hendrik *et al.,* 2015(Kamwati *et al.*, 2021). Meanwhile, the non-medical equipment including sink faucets, computer keyboards, and door handles were mostly colonized by *Pseudomonas aeruginosa* and CoN*S* (Rastogi *et al.*, 2012; Bhatta *et al.*, 2021; Alphons *et al.*, 2020).

1.2.6 Antimicrobial resistance in nosocomial infections

Resistant bacteria are fast spreading as causes of nosocomial infections. Out of the 76 positive cultures in the study by Ghafoor *et al.* (2020), 19 (25%) were caused by MRSA. The MRSA detected by Kumar *et al.* (2018) was also resistant to oxacillin, penicillin, erythromycin, and ciprofloxacin. Some of the *P. aeruginosa* isolated by Ghafoor *et al.* (2020) were resistant to several antibiotics tested including ampicillin, gentamicin, imipenem, meropenem, and ciprofloxacin. Kumar *et al.* (2018) also detected *P. aeruginosa* that were resistant against piperacillin. All the *E. coli* detected by Ghafoor *et al.* (2020) were resistant to ampicillin, amoxicillin/clavulanate, and trimethoprim/sulfamethoxazole. Some were also resistant to several other antibiotics such as gentamicin, imipenem, meropenem, cefoperazone/sulbactam, and ciprofloxacin (Ghafoor *et al.*, 2020). The *E. coli* isolated by Kumar *et al.* (2018) showed resistance against piperacillin, piperacillin-tazobactam, ceftazidime, and ciprofloxacin. The *A. baumanii* isolated by Kumar *et al.* (2018) showed resistance against piperacillin, piperacillin-tazobactam, amikacin, and netilmicin.

Regarding antimicrobial susceptibility of the bacteria isolated from cultures of swabs from fomites, most were highly sensitive to ceftriaxone, imipenem, and meropenem. However, MRSA, vancomycin-resistant *Enterococcus* (VRE) and multi-drug resistant Gram-negative *bacilli* (MDR GNB) have also been isolated (Marchant *et al.*, 2013; M. Okolo *et al.*, 2016; Dias *et a.,l* 2019; El-Sokkary *et al.,* 2019).

1.2.7 Rationale

In 2018, 2.5 million neonates died in the first twenty-eight days of life globally, which implies about 7,000 neonatal deaths every day (UNICEF, 2020). Neonatal deaths accounted for 47% of under-five mortality (UNICEF, 2020). Some of the diseases implicated in the high mortality rates include neonatal sepsis, bacteremia, pneumonia, and meningitis. The diseases

are mostly due to nosocomial infections (Ramasethu, 2017; Kweyu, Omwenga, and Maiyoh, 2021).

Various hospital inanimate objects have been implicated in nosocomial infections. Contaminated NICU surfaces, medical devices, and non-medical equipment are the primary reservoirs of bacteria that cause nosocomial infections (Hendrik *et al.,* 2015; Baek *et al.*, 2020b; Asinobi *et al.*, 2021; Kandwal *et al.*, 2019). As the reviewed articles show, different NBU settings have a unique composition of bacterial contaminants. For example, while gram-positive bacteria were the commonest causes of nosocomial infections in the study by Kweyu, Omwenga, and Maiyoh (2021), Ghafoor *et al.* (2020) reported gram-negative bacteria as the commonest causes of nosocomial infections in their facility. Therefore, detecting and characterizing the bacteria that could cause nosocomial infections in individual NBUs is crucial.

There is limited information on bacteria contamination of surfaces and equipment in NBU at KNH and their role as the source of infection to the preterm and term sick neonate infants. It is important to investigate the bacterial profile of the contaminants of equipment (medical and non-medical items) and surfaces and determine the antimicrobial susceptibility pattern of the potentially pathogenic bacteria on the equipment. The information can be used to review the infection prevention and control guidelines of KNH's NBU.

1.2.8 Study questions

1. What is the profile of bacteria isolated from surfaces and equipment in the newborn unit at Kenyatta National Hospital?
2. What is the antimicrobial susceptibility pattern of Coagulase negative staphylococci*, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* isolated from the newborn unit at Kenyatta National Hospital?

1.2.9 Study objectives

1.2.9.1 Main objective

To investigate the bacterial contaminant profile of surfaces and equipment in newborn unit at Kenyatta National Hospital and to determine the antimicrobial susceptibility pattern of

selected pathogenic bacteria including C*o*N*S, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*.

1.2.9.1 Specific objectives

1. To identify the bacteria that contaminate surfaces and equipment in the newborn unit at Kenyatta National Hospital.
2. To determine antimicrobial susceptibility pattern of the potentially pathogenic bacteria recovered from the surfaces namely CoN*S, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae Pseudomonas aeruginosa*.

Chapter 2: Methodology

2.1 Study design

The study was a cross-sectional study

2.2 Study area

The study was conducted in the Kenyatta National Hospital (KNH) Newborn Unit (NBU). The unit admits 200–300 neonates (babies aged up to one month at the time of admission) every month. NBU admissions come from KNH as well as other hospitals and the community. The unit has a few dozen incubators for pre-term babies and tens of cots for sick term babies. It has a seven-bed Neonatal Intensive Care Unit (NICU1), which has mechanical ventilators, radiant warmers, and Continuous Positive Airway Pressure (CPAP) machines. NICU2 has baby cots, oxygen pots, radiant warmers, and CPAP. There are nursery B1 and nursery B2 for babies less than 1600gms; it has incubators, oxygen pots, and radiant warmers. It has nurseryB3 for babies above 1600gms to 1800gms; its equipment includes baby cots, oxygen pots, and radiant warmers. Nursery D is also there for clinically stable babies.

The NBU has an isolation room for babies with a confirmed positive culture and referral babies from other hospitals and communities. The room has radiant warmers, baby cots, oxygen pots, and incubators. There is also an admission room with radiant warmers, CPAP, suction machine, and oxygen pots. A nurse station with tables, stools, chairs, computers, sink, and tap as shown in figure 1 is available in the NBU. Other rooms in the NBU include the milk preparation room, utility room, and equipment cleaning room. Each room in NBU/NICU has one table, chairs, sink, and tap.

The NBU is served by dozens of healthcare workers of various cadres – neonatologists, residents, medical officers, clinical officers, physiotherapists, nurses, and nutritionists. The newborn unit of KNH is in a referral and teaching hospital, hence it is overcrowded and has a high bed occupancy. Neonates share incubators, baby cots, and radiant warmers. The NBU is characterized by a high rate of health care worker activities. Cleaning of the floor in the NBU of KNH is done every 3 hours using sodium hypochlorite. Surfaces and equipment are cleaned and disinfected using dimethyl benzyl, ammonium chlorides 2.25%, dimethyl ethyl/

benzyl ammonium chlorides 2.25%, and multi-Tiered enzymatic detergent with rust inhibitors. Medical equipment is cleaned and disinfected weekly and after every discharge. If a patient is transferred, for instance from the incubator to a baby cot, the equipment is cleaned and disinfected. Health care providers follow standard handwashing protocol before and after examining each neonate as provided in IPC guidelines and standard operating procedures.

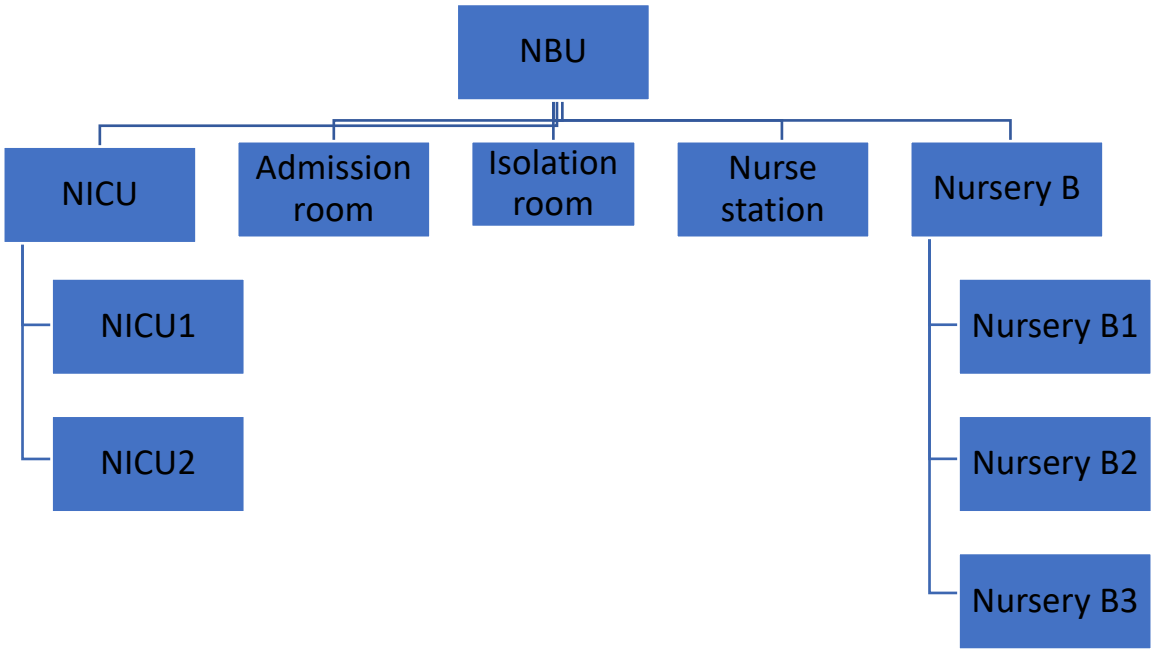

Figure 1. Newborn unit study area structure

NICU1 and NICU2 have babies who are on mechanical ventilation and nasal continuous positive airway pressure (NCPAP). NurseryB1and B2 have babies weighing less than 1600gms, very sick, and on incubators nursing. NurseryB3 has babies weighing 1600gms and above and on baby cots nursing if stable. The isolation room has babies with positive bacterial growth on blood culture or a referral from other health facilities. The nurse station is where patient notes are documented. The admission room has babies (referrals from other health facilities and KNH) with clinical conditions that need urgent interventions.

2.3 Study population

Medical equipment including incubators, oxygen masks, infant weighing scales, radiant warmers, baby cots, and suction machines will be swabbed. Non-medical equipment including PC keyboards, sinks, taps, PC mouses, door handles, and all the desk surfaces in NBU will be swabbed.

2.4 Inclusion criteria

Surfaces and equipment in the newborn unit.

2.5 Exclusion criteria

Surfaces and equipment not in use during sample collection.

2.6 Sample size

To determine the sample size, Fisher's formula was used (Israel, 2002). An assumed prevalence of 50% of all samples collected from equipment and environmental surface contaminated with bacteria was used to estimate the appropriate sample size. The 50% prevalence was used because literature is not consistent on the prevalence of the contamination and variance is maximum at 50% prevalence, hence the sample size is not underestimated.

$n = Z^2pq/d^2$ $\quad n = \dfrac{1.96^2 * 0.50\ (1-0.50)}{0.05^2} = 384$

Where:

n_0 = initial estimated sample study size

Z = standard normal deviate at 95% confidence interval (1.96)

p = estimated prevalence of bacteria isolated from contaminated surfaces and equipment in the newborn unit of KNH

q = 1-p

d = degree of freedom (0.05)

2.7 Variables

The independent variables included surfaces and equipment in the NBU. Dependent variables included isolated bacteria and antimicrobial susceptibility patterns of CoN*S, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa*.

2.8 Sampling procedure

Surfaces and equipment within the NBU that were in use and meeting the inclusion criteria were all sampled using sterile swabs. There was a disparity between different equipment in NBU. The swabbing of medical and non-medical equipment was systematically done once daily between 7 am to 10 am for seven weeks for the same surfaces and equipment until the required sample size was obtained. This was done by swabbing the surfaces and equipment of the predetermined areas with sterile cotton swabs dipped in normal saline (0.9% w/v).

The equipment was categorized into medical equipment including suction machines, oxygen masks, incubators, infant weighing scales, radiant warmers, and baby cots. Non-medical equipment includes taps, sinks, door handles, PC keyboards, PC mouses, and desk surfaces. Each equipment and desk surface in NBU was identified by its specific code (name, number, and location). For example, incubators 001 nursery B1, incubator 002 nursey B2, baby cots 001 nursery B2, and baby cot 002 nursery B3. Non-medical equipment was identified by its specific code also. Examples include door handle 001 admission room and sink 001 nursery B3. The desk surfaces in each section within NBU (DS 001 NICU1) were also coded accordingly. Each section was swabbed according to the equipment and desk surfaces present as shown in table 1.

All the swabs were labeled depending on their specific identification codes, location, and date of samples collected. They were then transported to the University of Nairobi Microbiology Laboratory within two hours of collection for microbiological analysis.

Table 1. Items swabbed from each room within NBU of KNH.

Medical equipment	Codes /location	Non-medical equipment	Code /location
Incubator	Incubator001NICU1	Sink	Sink001nurseryB2
Baby cot	Baby cot 001nurseryB1	Tap	Tap001nurseryB3
Oxygen mask	Oxygen mask001NICU2	Door handle	Door handl001NICU2
Radiant warmer	Radiant warmer001NICU1	PC keyboard	PC keyboard001nurse station
Suction machine	Suction machine001asmission room	PC mouse	PCmouse001surse station
Weighing scale	Weighing scale001nurseyB2		

2.8 Bacterial culture and identification

All the samples collected were cultured on MacConkey agar and blood agar. The inoculated plates were incubated at 37 °C for 24 hours and inspected after 24 hours of incubation. All bacteria isolated from culture-positive plates after 24 hours were identified by their colonial morphology, gram-staining, and biochemical tests which included catalase, indole, oxidase, Simmon's citrate utilization, and triple sugar iron as per the procedures outlined in appendix II.

2.9 Antimicrobial susceptibility test

Antimicrobial susceptibility testing of selected bacteria; CoN*S, Staphylococcus aureus, Escherichia coli, Klebsiella pneumoniae,* and *Pseudomonas aeruginosa* was performed on Mueller Hinton agar by using the Kirby-Bauer disk diffusion method. Samples collected were

analyzed and interpreted according to the 2017 Clinical and Laboratory Standards Institute (CLSIM100) guideline (CLSI., 2017). Selected antibiotics used include meropenem (10 µg), imipenem (10 µg), vancomycin (30 µg), clindamycin (2 µg), and penicillin (1IU) were tested for gram-positive bacteria. Gentamicin (30 µg), cefotaxime (30 µg), ceftazidime (30 µg), amikacin (30 µg), ceftriaxone (30 µg), clindamycin (2 µg), imipenem (10 µg), and meropenem (10 µg) were tested for gram-negative bacteria. *Pseudomonas aeruginosa* ATCC27853 and *E. coli* ATCC25922 were used in the identification and antimicrobial susceptibility testing as control strains (CLSI., 2017) as the procedure outlined in Appendix I.

2.10 Data management and analysis

Data generated were entered into Microsoft Excel and analyzed using Statistical Package for Social Sciences (SPSS) software version 21.0. Univariate analysis was done using frequency distributions and proportions for antimicrobial susceptibility patterns and isolated bacteria (categorical variables). In bivariate analysis, a Chi-square test was used to assess any significant association between bacteria spp isolated from surfaces and equipment in different NBU locations.

2.11 Ethical consideration

This proposal was approved by Kenyatta National Hospital and the University of Nairobi (KNH-UON) Ethics and Research Committee (P539/09/2020). Permission to conduct the study was sought from the Head, KNH pediatric and child health department, and Director UNITID. We obtained a waiver for the informed consent, as the study was not dealing with human subjects.

Chapter 3: Results

Five hundred and eight environmental surfaces and equipment swab cultures were collected from NBU. Swab cultures were taken from six different newborn unit locations/rooms (Admission room, isolation room, NICU (NICU1, NICU2, nurse station, nursery B (B1, B2, B3), and waiting area as shown in table 4. Sampling was done once per day using a sterile swab daily between 7 am to 10 am from February to March 2021. The number of swabs from medical and non-medical equipment that turned positive following culture is summarized in table 2. A total of 273/508 (54%) showed bacterial growth on culture, most were from medical equipment 178/308 (59%) and the rest from non-medical equipment 95/200 (48%).

Table 2. Distribution of bacteria growth culture in medical and non-medical equipment from the newborn unit

Equipment	Number of cultures (n = 508)	Bacterial isolation (n = 273)
Medical (n = 178)		
Cot	96	55 (57%)
Incubator	40	16 (40%)
Radiant warmer	88	51 (58%)
Weighing scale	6	4 (67%)
Suction machine	10	6 (60%)
Oxygen mask	68	46 (68%)
Non-medical (n = 95)		
Desk surface	57	29 (51%)
Door handle	44	17 (39%)
Keyboard	10	5 (50%)
PC mouse	11	4 (36%)
Sink	41	24 (59%)
Tap	37	16 (43%)

3.1 Bacterial isolation from surfaces and equipment in NBU

Among the bacteria isolated, CoN*S* were the most abundant 137/273 (50.2%) followed by *Klebsiella pneumoniae* 119/273 (43.6%) and *E. coli* 16/273 (5.9%) as shown in Figure 1.

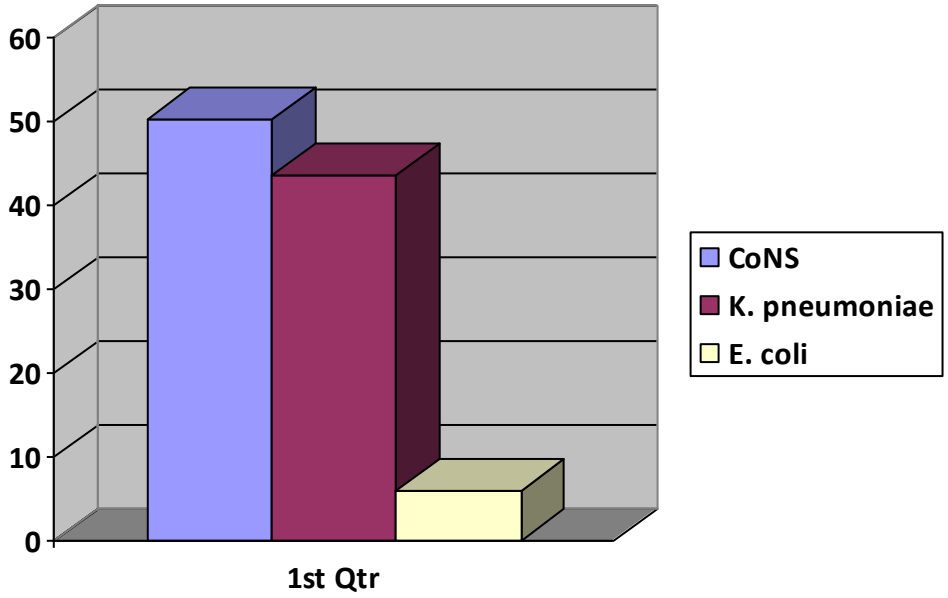

Figure 2. The proportion of bacteria isolated from environmental surfaces and equipment in the NBU.

3.2 Isolation of bacteria from different locations/rooms in the new-born unit

The majority of the positive bacterial cultures were from NICU1 and NICU2 as compared to other newborn unit locations/rooms and the differences were statistically significant ($p < 0.05$). Of positive cultures, 22% and 24% were *K. pneumoniae* and CoN*S* respectively as shown in Table 3.

Table 3. Bacteria isolated from different locations/rooms in the NBU

Site	Number of culture	CoN*S* n=137	*K. pneumoniae* n=119	*E. coli* n=16	*P. aeruginosa* n=1
NICU 2	60	33 (24%)	24 (20%)	3 (19%)	0 (0%)
NICU 1	48	17 (12%)	26 (22%)	5 (31%)	0 (0%)
Nursery B3	42	33 (24%)	9 (7%)	0 (0%)	0 (0%)
Nursery B1	32	15 (11%)	13 (11%)	4 (25%)	0 (0%)
Nursery B2	26	15 (11%)	9 (8%)	2 (13%)	0 (0%)
Admission room	22	7 (5%)	13 (11%)	2 (13%)	0 (0%)
Isolation room	21	5 (4%)	15 (13%)	0 (0%)	1 (100%)
Nurse station	19	11 (8%)	8 (7%)	0 (0%)	0 (0%)
Writing area	3	1 (1%)	2 (2%)	0 (%)	(0%)

3.3 Bacteria isolation from surfaces and equipment in NBU

Positive bacterial cultures were mostly isolated from surfaces of medical equipment (178/273, 65%) than non-medical equipment 95/273, 35%). However, the difference was not statistically significant (*p= 0.241*). Most of the positive bacterial cultures were from baby cots 55/273 (20%), radiant warmers 51/273 (19%), and oxygen masks 46/273 (17%). The least positive bacterial cultures were obtained from the baby weighing scales 4/273 (2%), PC mouses (2%), and the computer keyboards (2%) (p=0.03) as shown in table 4.

Table 4. Bacteria isolated from medical and non-medical equipment

Surface/equipment	Number of cultures (%)
Baby cots	55 (20.1%)
Radiant warmer	51 (18.7%)
Oxygen mask	46 (16.8%)
Incubator	16 (5.9%)
Suction machine	6 (2.2%)
Weighing scale	4 (1.5%)
Desk surface	29 (10.6%)
Sink	24 (8.8%)
Door handle	17 (6.2%)
Tap	16 (5.9%)
Keyboard	5 (1.8%)
PC mouse	4 (1.5%)

Most baby cots (55/273, 21%), NICU 1 (17/273, 30.9%), Nursery B3 (15/273, 27%), radiant warmer (25/273, 49%) and oxygen masks (16/273, 34%) from NICU 2 and incubators (9/273, 56%) in Nursery B1 were contaminated with bacteria. Other non-medical equipment contaminated with bacteria included desk surfaces in the nurse station (8/273, 28%), door handles (3/273, 17.6%) in NICU 2, admission room, and waiting area as shown in table 5.

Table 5. Isolation of bacteria from surfaces and equipment in NBU different locations/rooms

Equipment	Newborn Unit Site Positive Cultures (n = 273)								
	Admission room	Isolation Room	NICU		Nursing station	Nursery B			Waiting area
			1	2		1	2	3	
Cot	0 (0.0%)	5 (9.1%)	17 (30.9%	6 (10.9%	0 (0.0%)	6(10.9%	6 (10.9%)	15 (27.3%	0 (0.0%)
Radiant warmer	5 (9.8%)	3 (5.9%)	11 (21.6%	25 (49.0%	0 (0.0%)	4 (7.8%)	0 (0.0%)	3 (5.9%)	0 (0.0%)
Oxygen mask	7 (15.2%	0 (0.0%)	7 (15.2%	16 (34.8%	0 (0.0%)	2 (4.3%)	4 (8.7%)	10 (21.7%	0 (0.0%)
Incubator	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	9 (56.3%	6 (37.5%	1 (6.3%)	0 (0.0%)
Suction machine	0 (0.0%)	1 (16.7%	1 (16.7%	2 (33.3%	0 (0.0%)	2 (33.3%	0 (0.0%)	0 (0.0%)	0 (0.0%)
Weighing scale	2 (50.0%	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (25.0%	0 (0.0%)	1 (25.0%	0 (0.0%)
Desk surface	3 (10.3%	4 (13.8%	4 (13.8%	3 (10.3%	8 (27.6%	1 (3.4%)	4 (13.8%	2 (5.9%)	0 (0.0%)
Sink	1 (4.2%)	4 (16.7%	3 (12.5%	3 (12.5%	1 (4.2%)	5 (20.8%	2 (8.3%)	5 (20.8%	0 (0.0%)
Door handle	3 (17.6%	1 (5.9%)	2 (11.8%	3 (17.6%	0 (0.0%)	1 (5.9%)	2 (11.8%	2 (11.8%	3 (17.6%
Tap	1 (6.3%)	3 (18.8%	3 (18.8%	2 (12.5%	1 (6.3%)	1 (6.3%)	2 (12.5%	3 (1.8.8%	0 (0.0%)
Key board	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	5 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
PC mouse	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	4 (100%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	0 (0.0%)

CoNS and *K. pneumoniae* were the predominant bacteria isolated from different equipment. However, there was no significant difference in isolation of CoN*S* and *K. pneumoniae* from the equipment (p= 0.865) (Table 6).

Table 6. The proportion of bacteria species isolated from medical and non-medical equipment

Equipment	Bacteria			
	CoN*S* n=137/273	*E. coli* n=16/273	*K. pneumoniae* n=119/273	*P. aeruginosa* n=1/273
Baby cot (n=55)	28 (50.9%)	3 (5.5%)	24 (43.6%)	0 (0.0%)
Radiant warmer (n=51)	26 (51.0%)	4 (7.8%)	21 (41.2%)	0 (0.0%)
Oxygen mask (n=46)	27 (58.7%)	2 (4.3%)	17 (37.0%)	0 (0.0%)
Incubator (n=16)	8 (50.0%)	1 (5.9%)	7 43.8%)	0 (0.0%)
Suction machine (n=6)	4 (66.7%)	1 (16.7%)	1 (16.7%)	0 (0.0%)
Weighing scale (n=4)	2(50.0)	1(25.0%)	1(25.0%)	0(0.0%)
Desk surface (n=29)	15 (51.7%)	2 (6.9%)	12 (41.4%)	0 (0.0%)
Sink (n=24)	12 (50.0%)	0 (0.0%)	11 (45.8%)	1 (4.2%)
Door handle (n=17)	5 (29.4%)	1 (5.9%)	11 (64.7%)	0 (0.0%)
Tap (n=16)	6 (37.5%)	1 (6.3%)	9 (56.3%)	0 (0.0%)
PC Keyboard (n=5)	3 (60.0%)	0 (0.0%)	2 (40.0%)	0 (0.0%)
PC mouse (n=4)	1(25.0%)	0 (0.0%)	3 (75.0%)	0 (0.0%)

Abbreviation; n=number of colonies (Odoyo *et al*., 2021)

3.4 Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing results indicated that the grown bacteria cultures (n = 273/509) from NBU environmental surfaces and equipment showed high susceptibility to meropenem (90-100%), imipenem (93-100%), and amikacin (68-100%) for both gram-positive and gram-negative bacteria. Resistance was noted to clindamycin (46-100%), penicillin (97-100%), and vancomycin (45-100%) for gram-positive bacteria while gram-negative bacteria were resistant to clindamycin as outlined in Table 7.

CoN*S* were highly susceptible to meropenem (96%), amikacin (77%), and imipenem (93%). Resistance was recorded for vancomycin (45%), ceftazidime (48%), penicillin (97%), and clindamycin (46%). *K. pneumoniae* isolates showed high resistance to clindamycin (87%) but

high susceptibility to meropenem (90%), imipenem (97%), amikacin (68%), and ceftazidime (67%). Although *E. coli* and *P. aeruginosa* cultures were less than 30; the recommended CLSI threshold, we reported the AST results. Both were highly susceptible to meropenem (94-100%), amikacin (69-100%), and imipenem (94-100%) but they were resistant to clindamycin (100%). Cultures of swabs obtained from the different NBU environmental surfaces and equipment were highly susceptible to meropenem, amikacin, and imipenem (70-100%) but resistant to clindamycin, penicillin, and vancomycin (45-100%) as shown in table 7.

Table 7. Antimicrobial susceptibility profiles

Antibiotics		Bacterial species				
		N	CoN*S* n-137	*E. coli* n=16	*K. pneumonia* n=119	*P. aeruginosa* n=1
Meropenem						
	S	254	96%	94%	90%	100%
	I	11	2%	0%	7%	0%
	R	8	2%	6%	3%	0%
Amikacin						
	S	198	77%	69%	68%	100%
	I	51	16%	25%	21%	0%
	R	24	7%	6%	11%	0%
Clindamycin						
	S	85	51%	0%	13%	0%
	I	4	3%	0%	0%	0%
	R	184	46%	100%	87%	100%
Penicillin G						
	S	2	1%	ND	ND	ND
	I	2	1%	ND	ND	ND
	R	269	97%	ND	ND	ND
Gentamicin						
	S	122	36%	69%	51%	0%
	I	75	32%	13%	24%	0%
	R	76	31%	19%	24%	100%
Cefotaxime						
	S	116	34%	44%	52%	0%
	I	105	40%	44%	36%	0%
	R	52	26%	13%	12%	100%
Ceftriaxone						
	S	124	30%	31%	66%	0%
	I	97	40%	63%	27%	0%
	R	52	30%	6%	8%	100%
Vancomycin						
	S	63	39%	ND	ND	ND
	I	23	16%	ND	ND	ND
	R	187	45%	ND	ND	ND
Imipenem						
	S	259	93%	94%	97%	100%
	I	7	2%	6%	3%	0%
	R	7	4%	0%	1%	0%
Ceftazidime						
	S	137	32%	81%	67%	0%
	I	50	20%	19%	17%	0%
	R	86	48%	0%	16%	100%

ND; Not determined

Chapter 4: Discussion

This study was conducted to determine the bacterial profile of contaminated surfaces and equipment and antimicrobial susceptibility patterns of *Klebsiella pneumoniae, coagulase-negative staphylococci (*CON*S), Escherichia coli, Staphylococcus aureus,* and *Pseudomonas aeruginosa* in the newborn unit at Kenyatta National Hospital.

The overall bacterial contamination of surfaces and equipment (54%) found in this study is similar to published studies that have reported contamination rates ranging from 52.8% to 74.6% in NICU (Alphons *et al.*, 2020; Bhatta *et al.*, 2021). The high bacterial contamination of surfaces and equipment in NBU/NICU observed may be attributed to overcrowded units, high bed occupancy (neonates share incubator, baby cot, and radiant warmer), neonates admitted with different clinical conditions from different health facilities, and poor compliance to infection control practices as previously described (Bhatta *et al.*, 2021). This might indicate that healthcare workers, parents, and visitors could be a source of infection to the NBU/NICU environment because of interactions that might help in the spread and transmission of infections (Alphons *et al.*, 2020). Additionally, occasional disinfection of surfaces and equipment within the hospital settings may facilitate microbial colonization, growth, and survival; consequently increasing the risk of infections in a susceptible neonate.

The predominant bacteria isolated from the newborn unit environmental surfaces and equipment in this study were Co*NS* followed by *K. pneumoniae* and *E. coli.* Most previous studies have isolated similar patterns of bacteria from NBU/NICU surfaces and equipment (El-Sokkary, Hassanein, and Elsayed, 2019; Dias and Saleem, 2019). Notably, *Staphylococci aureus* was not isolated in this study despite its sporadic involvement in causing nosocomial infections (Bhatta et al., 2018). A possible explanation for isolation reduction could be due to compliance with infection control and prevention interventions implemented by the NBU.

CoN*S* were the major bacterium isolated from newborn unit surfaces and equipment. The finding is consistent with the results of studies in Namibia and Egypt that showed CoN*S* accounting for the majority of bacteria isolated from NBU/NICU inanimate and environmental surfaces (Alphons *et al.*, 2020; Dias and Saleem, 2019). CoN*S* are mainly considered colonizers of the human body and skin and can be spread by the hands of healthcare workers, parents, and guardians who come to visit their sick neonates. A high level of contamination of surfaces and equipment mainly medical equipment in NBU indicates that

there is negligence of hand hygiene practice in NBU. Therefore, there is need for thorough disinfection, use of appropriate disinfectants, and application of updated infection control practices to minimize the spread of infections in NBU (Alphons *et al.*, 2020).

Other potential pathogens isolated were *K. pneumoniae, Escherichia coli,* and *Pseudomonas aeruginosa*. *Enterobacteriaceae* and *P. aeruginosa* are known colonizers of the large intestine. They can be shed into the hospital environment, consequently causing a large percentage of nosocomial infections. Moreover, their persistence in the hospital environment is attributed to resistance to commonly used disinfectants and antibiotics, hence posing a risk of difficult-treat infections to neonates.

The level of contamination differed from one item to another. Baby cots, desk surfaces, oxygen masks, and radiant warmers showed high levels of bacterial contamination as reported in previous studies (Bhatta *et al.*, 2021; (Rastogi *et al.*, 2012). Ambient humidity and temperature levels of some items like radiant warmers are ideal for the survival of pathogenic bacteria. Furthermore, contamination of these items could be linked to frequent hand contact of the healthcare workers, visitors, or patients. Contamination of these items in NICU/NBU generally poses a risk of disease transmission to neonates and therefore strict adherence to disinfection guidelines of high touch areas in NICU/NBU is crucial.

Concerning the antimicrobial susceptibility of the bacteria isolates recovered in this study, the majority showed high susceptibility to meropenem, imipenem, amikacin, and ceftazidime. resistance was noted in penicillin, vancomycin, and clindamycin. The results in this study are similar to a study done in Nigeria in which all isolates were susceptible to meropenem (M. O. Okolo *et al.*, 2016). In another study in Egypt, all isolates were sensitive to imipenem and resistant to cefotaxime (El-Sokkary, Hassanein, and Elsayed, 2019) while in Namibia most isolates showed high resistance to penicillin and cephalosporins (Alphons *et al.*, 2020). The resistance observed could be attributed to selective pressure arising from the frequent use of these antibiotics in the treatment of the infections caused by these bacterial pathogens.

Interestingly, CoN*S* recorded a high resistance level to vancomycin regardless of limited use in treating CoN*S* related diseases. However, CLSI recommends confirming such isolates with a minimum inhibitory concentration results of a broth dilution test. Therefore, the results from this study should be interpreted with caution considering that the disk diffusion method was used (CLSI, 2017). Overall, the presence of the resistant bacterial agents in NICU/NBU

poses a public health concern that needs urgent interventions in reducing nosocomial disease incidences in neonates.

The findings of this study have provided baseline information on the bacteria that contaminate NBU/NICU surfaces and equipment at KNH. It also revealed the resistance patterns of some pathogenic bacteria isolated from the contaminated surfaces and equipment.

4.1 Limitation

This study isolated only bacterial microorganisms and yet the newborn environment could be contaminated with other potential pathogens including parasites, fungi, and viruses. Future studies should investigate the role of other bacterial agents as well as fungal isolates from NICU/NBU in the development of neonatal infections. Stratified sampling would have been more representative than the systematic sampling that was used for sample collection.

4.2 Conclusion

This study identified bacterial contamination of surfaces and equipment in which CONS*, K. pneumoniae,* and *E. coli* were the predominant bacteria isolated from desk surfaces and medical equipment more than non-medical equipment. The majority of bacteria isolated were sensitive to meropenem, imipenem, and amikacin and were resistant against penicillin, clindamycin, and vancomycin.

4.3 Recommendation

Potentially pathogenic bacteria like CoN*S*, *K. pneumoniae,* and *E. coli* contaminating desk surfaces and medical equipment in the NBU are a threat to neonates. This study recommends the following to minimize the spread of potential pathogenic bacteria in the newborn unit at Kenyatta National Hospiital:

- Control in the number of visitors to NBU
- Adherence to infection prevention practices
- Improvement or reduction of the bed occupancy if possible
- Appropriate use of disinfectants in decontaminating high touch areas

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APPENDICES

Appendix 1 Antimicrobial sensitivity testing using disk diffusion method

Kirby-Bauer disk diffusion test is used to determine the sensitivity or resistance of isolated bacteria. The bacterium is grown on Mueller-Hinton agar in the presence of antimicrobial impregnated filter paper disks. When a filter paper disk is impregnated with an antimicrobial on Mueller-Hinton agar, immediately water is absorbed into the disk from the agar, and antimicrobial begins to diffuse into the surrounding agar, therefore the concentration of antimicrobial is highest closest to the disk and a reduction in concentration occurs as the distance from the disk increases.

Appendix II Microbiological tests

Gram stain

Used to identify gram-positive (appears purple) and gram-negative bacteria (appears pink)

1. A smear is prepared by emulsifying the specimen/colony on a drop of normal saline

2. Air dried and fixed by passing over a flame three times

3. Covered with the primary stain; crystal violet stain for 1 minute

4. Wash off the stain with clean water

5. Drain the water and flood the smear with grams iodine for 1 minute

6. Wash off the gram's iodine with clean water

7. Decolorize the smear with acetone alcohol and rinse immediately with clean water

8. Cover the smear with neutral red stain for 1 minute and rinse with clean water

9. Wipe the back of the slide using cotton soaked in 70% alcohol and blot dry

10. Examine microscopically using oil immersion objective for cells

Colonies morphology

1. **MacConkey Agar:** It is a selective and differential medium. The pH indicator helps to differentiate between lactose fermenting and Lactose non-fermenter. *E. coli* and other lactose fermenting bacteria give pink-colored colonies in MacConkey agar whereas non-lactose fermenter gram-negative bacilli produce pale yellow colonies.
2. **Blood Agar**: usually produce non-hemolytic smooth white colonies

Biochemical test characteristics

Catalase test

This test is used to differentiate the bacteria that produce the enzyme catalase such as staphylococci from non-catalase-producing bacteria such as streptococci.

Method

I. 2-3 of hydrogen peroxide solution will be poured into a test tube
II. Using a wooden stick or a glass rodes several colonies of the test bacteria are removed and immersed in the hydrogen peroxide solution
III. Active bubbling induces a positive catalase test

Coagulase test

This test is used to identify Staphylococcus aureus which produces coagulase. Both tube test and slide test are employed

Method

Slide test (detects bond coagulase)

I. A drop of distilled water will be placed on each end of a slide or two separate slides
II. A colony of the test tube will be emulsified in each of the drops to make two thick suspensions.
III. A loop full (not more than) will be added on one of the suspensions and mixed gently.
IV. Clumping of the bacteria will occur within 10seconds if the bacteria is *Staphylococcus aureus*.

V. No plasma is added to the second suspension. This is used to differentiate any granular appearance of the bacteria from true coagulase clumping.

Test tube (detects free coagulase)

I. Plasma will be diluted in the ratio of 1:10
II. Three small test tubes will be availed and labeled; test bacteria, positive control, and negative control.
III. 0.5ml of diluted plasma will be pipetted into each tube
IV. Five drops (about 0.1ml) of the test bacteria will be added into the labeled positive, and 5drops of the Staphylococcus aureus culture will be added to the tube labeled positive, and 5drops of sterile broth in the tube labeled negative
V. The tubes will be incubated at 35-37ºc after mixing gently. Clotting will occur after one hour if no clotting occurs after one-hour examination will be repeated after every 30minutes for up to 6hours.
VI. Clotting is indicative of Staphylococcus aureus

Oxidase test

This test is used to identify *Pseudomonas*

Method

1. A piece of filter paper will be placed in a petri dish and soaked with 2-3 drops of freshly prepared oxidase reagent.
2. Using a piece of stick or glass rod, a colony of the test bacteria will then be smeared on the filter paper.
3. The development of blue-purple color within a few seconds indicates a positive oxidase test.

Voges-proskeur (VP) test

This test is used to test *klebsiella spp*

Method.

I. 2ml of sterile glucose phosphate peptone water will be inoculated with the test bacteria and incubation at 35-37ºc for 48hours.
II. A small amount of creatinine will be added and mixed well
III. 3ml of sodium hydroxide will be added and mixed well
IV. The bottle cap will be removed and left for one hour at room temperature
V. The development of pink color will be indicative of *Klebsiella Pneumonia*.

Citrate utilization test

Citrate utilization test is commonly employed as part of a group of tests, the IMViC (Indole, Methyl Red, VP and Citrate) tests, that distinguish between members of the *Enterobacteriaceae* family

Procedure of citrate utilization test:

1. Inoculate Simmons citrate agar on the slant by touching the tip of a needle to a colony that is 18 to 24 hours.
2. Incubate at 35ºC to 37ºC for 18 to 24 hours.
3. Observe the development of blue color.

-Citrate positive: visible blue color on the slant surface e.g., *Klebsiella pneumoniae*

-Citrate negative: no visible growth or no color change e.g., *Escherichia coli*

Indole test

This test is used to differentiate *Enterobacteriaceae*

Procedure of indole test

a Inoculate the isolated colony in tryptophan broth.
b Incubate at 37°C for 24-28 hours in ambient air.
c Add 0.5 ml of Kovac's reagent to the broth culture.

Positive: Pink colored e.g*., E. coli*

Negative: No color change e.g., *Klebsiella pneumoniae*

Triple sugar iron

This test is used to differentiate *Enterobacteriaceae* that ferment lactose. It's a test which has three sugar (Lactose, Sucrose, and Glucose) and also iron; and it contains Agar as solidifying agent (TSI is a semi solid media having slant and butt).

Procedure for Triple Sugar Iron Agar (TSI) Test

1. With a sterilized straight inoculation needle touch the top of a well-isolated colony
2. Inoculate TSI Agar by first stabbing through the center of the medium to the bottom of the tube and then streaking on the surface of the agar slant.
3. Leave the cap on loosely and incubate the tube at 35°C in ambient air for 18 to 24 hours

Interpretation of Triple Sugar Iron Agar Test

1. Alkaline slant/no change in butt (K/NC) i.e., Red/Red = glucose, lactose and sucrose non-fermenter
2. Alkaline slant/Alkaline butt (K/K) i.e., Red/Red = glucose, lactose and sucrose non-fermenter
3. Alkaline slant/acidic butt (K/A); Red/Yellow = glucose fermentation only, gas (+ or -), H2s (+ or -)

4. Acidic slant/acidic butt (A/A); Yellow/Yellow = glucose, lactose and/or sucrose fermenter gas (+ or -), H2s (+ or -).

Bacteria	**Slant**	**Butt**	**Gas**	**H2S**
Escherichia, Klebsiella,	Acid (A)	Acid (A)	Pos (+)	Neg (-)

Appendix III Ethical approval from UON/KNH-ERC

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Ref: KNH-ERC/A/20

21 January 2021

KENYATTA NATIONAL HOSPITAL APPROVED 21 JAN 2021 KNH/UoN-ERC P. O. BOX 20723 - 00202 NAIROBI

Dr. Adut Chan Malual
Reg. No.W64/6778/ 2017
Institute of Tropical and Infectious Diseases(UNITID)
College of Health Sciences
University of Nairobi

Dear Dr. Malual

RESEARCH PROPOSAL – BACTERIAL CONTAMINATION OF SURFACES AND EQUIPMENT AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF POTENTIALLY PATHOGENIC BACTERIA IN NEWBORN UNIT AT KENYATTA NATIONAL HOSPITAL (P539/09/2020)

This is to inform you that the KNH- UoN Ethics & Research Committee (KNH- UoN ERC) has reviewed and **approved** your above research proposal. The approval period is 21st January 2021 – 20th January 2022.

This approval is subject to compliance with the following requirements:

a. Only approved documents (informed consents, study instruments, advertising materials etc) will be used.
b. All changes (amendments, deviations, violations etc.) are submitted for review and approval by KNH-UoN ERC before implementation.
c. Death and life threatening problems and serious adverse events (SAEs) or unexpected adverse events whether related or unrelated to the study must be reported to the KNH-UoN ERC within 72 hours of notification.
d. Any changes, anticipated or otherwise that may increase the risks or affect safety or welfare of study participants and others or affect the integrity of the research must be reported to KNH- UoN ERC within 72 hours.
e. Clearance for export of biological specimens must be obtained from KNH- UoN ERC for each batch of shipment.
f. Submission of a request for renewal of approval at least 60 days prior to expiry of the approval period. (*Attach a comprehensive progress report to support the renewal*).
g. Submission of an *executive summary* report within 90 days upon completion of the study. This information will form part of the data base that will be consulted in future when processing related research studies so as to minimize chances of study duplication and/ or plagiarism.

For more details consult the KNH- UoN ERC website**http://www.erc.uonbi.ac.ke**

Yours sincerely,

PROF. M. L. CHINDIA
SECRETARY, KNH-UoN ERC

c.c. The Principal, College of Health Sciences, UoN
The Senior Director, CS, KNH
The Chairperson, KNH- UoN ERC
The Assistant Director, Health Information Dept, KNH
The Director, Institute of Tropical and Infectious Diseases (UNITID), UoN
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Date: 1st February, 2021

Dr. Adut Chan Malual
Institute of Tropical and Infectious Diseases (UNITID)
College of Health Sciences
University of Nairobi

Dear Dr. Adut

RE: AUTHORITY TO COLLECT DATA IN PAEDIATRICS DEPARTMENT

Following approval by the KNH/UON-Ethics & Research Committee for your Research Proposal and subsequent filing of the Study Registration Certificate, this is to inform you that authority has been granted to collect data in ***Paediatrics Department, Newborn Unit*** on your study titled ***"Bacterial contamination of surfaces and equipment and antimicrobial susceptibility pattern of potentially pathogenic bacteria in Newborn Unit at Kenyatta National Hospital"***.

Kindly liaise with the Senior Assistant Chief Nurse Paediatrics for facilitation.

Kindly submit a report of your study findings to the office of the undersigned after completion of your study.

Dr. Douglas Makewa
HEAD OF DEPARTMENT, PAEDIATRICS

Cc. Senior Assistant Chief Nurse, Paediatrics
Assistant Chief Nurse Incharge, Newborn Unit

KNH/R&P/FORM/01

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Study Registration Certificate

1. Name of the Principal Investigator/Researcher
Dr. Adut Chan Malual

2. Email address: adut8s@yahoo.com Tel No. 0701828155

3. Contact person (if different from PI) Achan Chan Malual

4. Email address: Tel No. 0721929952

5. Study Title
Bacterial Contamination of Syringes and equipment and antimicrobial susceptibility pattern of potentially pathogenic bacteria in newborn unit at Kenyatta National Hospital

6. Department where the study will be conducted Paeds
(Please attach copy of Abstract)

7. Endorsed by KNH Head of Department where study will be conducted.

Name: [illegible] Signature [signature] Date 1/2/21

8. KNH UoN Ethics Research Committee approved study number _____
(Please attach copy of ERC approval)

9. I Adut Chan Malual commit to submit a report of my study findings to the Department where the study will be conducted and to the Department of Medical Research.

Signature [signature] Date 29/01/2021

10. Study Registration number (Dept/Number/Year) Paeds /264/2021
(To be completed by Medical Research Department)

11. Research and Program Stamp KENYATTA NATIONAL HOSPITAL 05 FEB 2021 Research & Programs Resource Centre

All studies conducted at Kenyatta National Hospital **must** be registered with the Department of Medical Research and investigators **must commit** to share results with the hospital.